Cytoskeleton in motion: the dynamics of keratin intermediate filaments in epithelia

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Epithelia are exposed to multiple forms of stress. Keratin intermediate filaments are abundant in epithelia and form cytoskeletal networks that contribute to cell type–specific functions, such as adhesion, migration, and metabolism. A perpetual keratin filament turnover cycle supports these functions. This multistep process keeps the cytoskeleton in motion, facilitating rapid and protein biosynthesis–independent network remodeling while maintaining an intact network. The current challenge is to unravel the molecular mechanisms underlying the regulation of the keratin cycle in relation to actin and microtubule networks and in the context of epithelial tissue function.

Introduction

Protecting tissues and organs against ever-changing environmental challenges is a major function of epithelial tissues. Renewal and repair of epithelia involve continuous cycles of proliferation, migration, and differentiation. As a result, the epithelial cytoskeleton is perpetually remodeled to optimize epithelial functions in space and time. The most diverse and abundant cytoskeletal components of epithelial cells are keratin intermediate filaments (KFs); there are >50 isotypes expressed in epithelia (Schweizer et al., 2006; Moll et al., 2008; Bragulla and Homberger, 2009). Recently identified regulatory functions of keratins in organelle trafficking, motility, translation, signaling, immune response, and cell survival indicate that KFs have the plasticity and network architecture to fine-tune epithelial function (Toivola et al., 2005; Kim et al., 2006, 2007; Long et al., 2006; Kim and Coulombe, 2007; Magin et al., 2007; Vijayaraj et al., 2009; Depianto et al., 2010; Ku et al., 2010). One of the key questions is how keratins provide rigidity and strength but at the same time remain dynamic and flexible. At present, the molecular mechanisms governing keratin assembly, disassembly, and network architecture are largely unknown.

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Abbreviations used in this paper: IF, intermediate filament; KF, keratin IF; ULF, unit length filament.

We will discuss properties of a biosynthesis-independent multistep assembly/disassembly cycle of keratins that allows rapid network remodeling without network disruption.

Time-lapse imaging of cultured monolayers of living cells producing fluorescent keratins revealed that the network is highly dynamic (Windoffer and Leube, 1999; Yoon et al., 2001; Windoffer et al., 2004). These observations implicate a perpetual cycle of KF assembly and disassembly in a conventional (2D) culture setting (Figs. 1 and 2 and Video 1; Kölsch et al., 2010; Leube et al., 2011). In brief, the cycle begins with nucleation of keratin particles at the cell periphery, often in close proximity to lamellipodial focal adhesions. This is followed by elongation of newly formed keratin particles during actin-dependent translocation toward the peripheral keratin network. After integration of precursor particles into the network, KFs continue to move toward the nucleus and bundle. Some of them disassemble into soluble oligomers that rapidly diffuse throughout the cytoplasm and are available for another round of nucleation in the cell periphery. Others mature into a stable network that surrounds the nucleus and is anchored to desmosomes and hemidesmosomes. Collectively, cycling allows the epithelial cytoskeleton to remain in motion without loss of structural integrity.

Keratin network assembly

In cultured epithelial cells, formation of KFs, referred to as nucleation, starts in the cell periphery in close vicinity to focal adhesions (Windoffer et al., 2006). Focal adhesions anchor actin bundles (Petit and Thiery, 2000; Geiger et al., 2001; Carragher and Frame, 2004) and induce changes in microtubule network architecture (Krylyshkina et al., 2003; Small and Kaverina, 2003). By also linking KF nucleation to these sites, coordinated restructuring of the entire cytoskeleton is accomplished. This is of particular relevance for moving cells. In accordance, a remarkable increase of keratin particle formation is observed in lamellipodia of migrating cells (Wöll et al., 2005; Kölsch et al., 2010; Rolli et al., 2010). A possible nucleus for filament formation has been identified in vitro: the ∼60-nm-long unit length filament

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Figure 1. **The keratin cycle.** Soluble keratin oligomers assemble into particles in the cell periphery in proximity to focal adhesion sites (nucleation). These particles grow (elongation) and move toward the cell center in an actin-dependent process (transport). Subsequently, elongated KF particles are incorporated into the peripheral KF network (integration). Filament bundling occurs during further centripetal translocation toward the nucleus (transport). Soluble oligomers dissociate (disassembly), diffuse throughout the cytoplasm (diffusion), and are reutilized for another cycle of KF formation in the cell periphery. Alternatively, bundled filaments are stabilized (maturation), forming, e.g., the stable perinuclear cage.
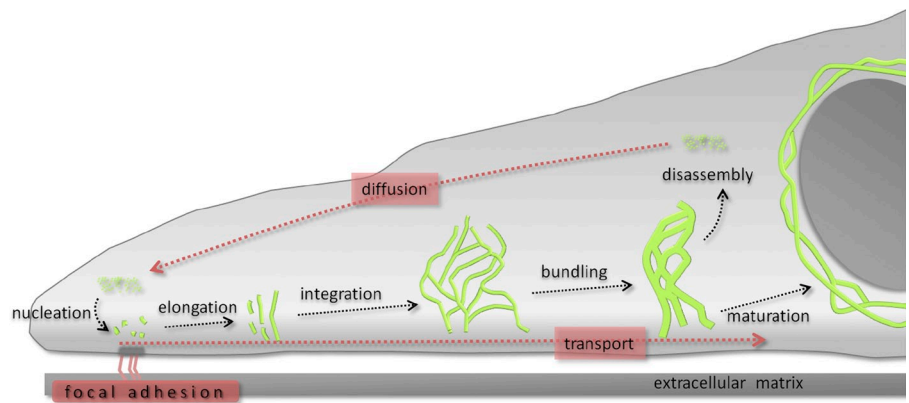
(ULF), which consists of 32 monomers (Herrmann et al., 1999; Herrmann et al., 2002). In living cells, it is not clear whether the particles that can form KFs, termed KF precursors (Windoffer et al., 2004), are the same as ULFs because the resolution of standard light microscopy cannot distinguish single ULFs.

Because of the lack of KF precursor polarity, both ends are equally suited to support elongation by oligomer addition. In vitro observations of vimentin intermediate filaments (IFs) suggest that single and multiple ULFs are indiscriminately added at either end (Kirmse et al., 2007). In accordance with these findings, live cell imaging of keratins reveals continuous particle elongation and fusion of larger particles (Windoffer et al., 2004, 2006; Wöll et al., 2005). As long as they retain their free ends, they elongate. When particles approach the filament network, they integrate via their ends, thereby adding another branch to the filament network (Windoffer et al., 2004, 2006; Wöll et al., 2005). In the case of mutant keratins that cause blistering skin diseases in humans, elongated filaments are not formed, and, instead, short-lived spheroidal granules are generated near focal adhesions (Werner et al., 2004; Windoffer et al., 2006).

Keratin networks are heterogeneous; they are composed of 2–10 different isotypes. The modes and mechanisms governing their organization and distribution remain unknown but may depend to a considerable extent on the degree of cell polarity. The concept that the intracellular distribution of keratin isotypes results from their primary sequence is intriguing but not well-supported by existing data. In intestinal epithelia of the mouse, the isotypes K20 and K8 are codistributed throughout the cell, whereas in umbrella cells of the bladder, K20 is restricted to the apical domain, which suggests cell type–specific mechanisms (Magin et al., 2006). Another feature of network organization is coexistence of individual filaments and bundles (i.e., interfilament assemblies). Consequences of bundling are increased mechanical stability and reduced turnover (Flitney et al., 2009; Lee and Coulombe, 2009; Kim et al., 2010), prerequisites for a resilient and durable cytoskeletal scaffolding. However, this may not always be the case because in the absence of the cytoskeletal cross-linker plectin, bundling is even increased but appears to be dysfunctional because cellular resilience is reduced (Osmanagic-Myers et al., 2006). In cultured cells, bundling is reflected by increasing KF diameter toward the nucleus caused by lateral association of filaments (Windoffer et al., 2004; Lee and Coulombe, 2009; Kölsch et al., 2010).

Different factors influence bundling: (a) IF-associated proteins (Krieg et al., 1997; Xu et al., 2000; Makino et al., 2001; Listwan and Rothnagel, 2004; Long et al., 2006; Osmanagic-Myers et al., 2006; Boczonadi et al., 2007; Ishikawa et al., 2010); (b) intrinsic and isotype-specific properties of KFs themselves (Eichner et al., 1986; Blessing et al., 1993; Hofmann et al., 2000), which bundle spontaneously in vitro in a process referred to as self-organization (Lee and Coulombe, 2009; Kim et al., 2010); and (c) phosphorylation, which has been shown to coincide with bundling upon mechanic and chemical stress (Strnad et al., 2001; Flitney et al., 2009).

Keratin filament disassembly

Because KF assembly is highly favored over disassembly, mechanisms must exist to remove assembled filaments and especially dense filament bundles that would interfere with cellular functions. Possible mechanisms regulating this balance are degradation of KF polypeptides and/or disassembly of KFs into reusable subunits. In support of the first mechanism, ubiquitination of keratins and subsequent proteasomal degradation has been described previously (Ku and Omary, 2000; Löffek et al., 2010; Rogel et al., 2010). It is elevated in stress and pathology, which is presumably a consequence of increased network restructuring (Zatloukal et al., 2007; Jaitovich et al., 2008; Na et al., 2010). This aspect has been exploited to reduce aggregates typical of keratinopathies by application of chemical chaperones and chaperone-associated ubiquitin ligases (Lee et al., 2008; Chamcheu et al., 2010; Löffek et al., 2010). The second mechanism, however, seems to be the major mode in rapidly dividing cultured cells, as time-lapse fluorescence recordings showed that KF formation occurs independent of and in the absence of protein biosynthesis (Windoffer et al., 2004; Kölsch et al., 2010). Imaging of single inward-moving KF bundles further revealed that they dissolve over time without appearance of distinct fragments, which indicates that the released subunits are nonfilamentous (Fig. 2 and Video 1). Mechanistically, the release of soluble subunits may occur similarly to the lateral subunit exchange that has been described for IFs at equilibrium (Eriksson et al., 2009). At present, although it is not known how disassembly is regulated, the involvement of phosphorylation is likely, as inhibition of p38 MAPK or PKCζ activities induces increased network stability and, conversely, increased kinase activities result in enhanced KF network
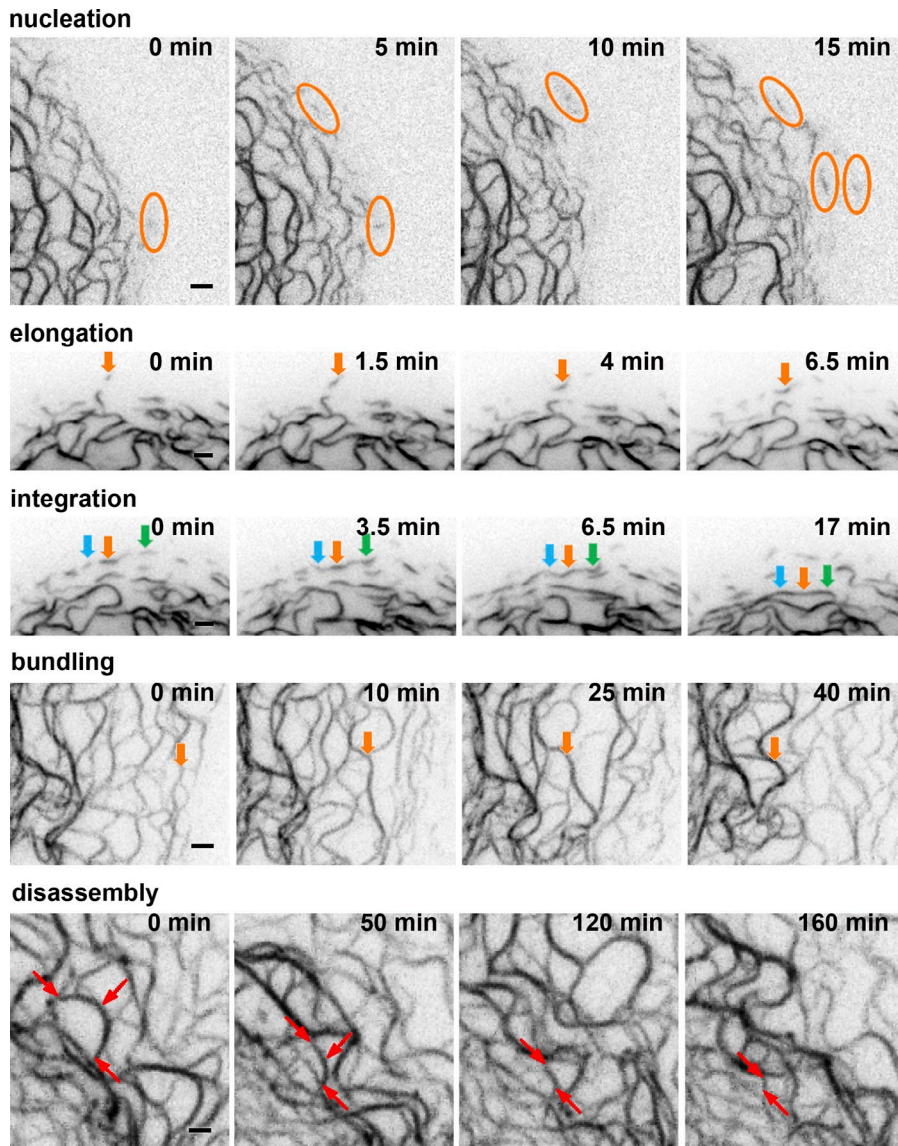
Figure 2. **Steps of the keratin cycle.** Representative images (inverse presentation), taken from time-lapse fluorescence microscopy of cultured cells producing fluorescent keratins (see Video 1), that illustrate the steps of the keratin cycle (see also Fig. 1). Images were adapted with permission from the *Journal of Cell Science* (Kölsch et al., 2010) and *Molecular Biology of the Cell* (Windoffer et al., 2004). Nucleation: Newly appearing particles that are generated in proximity to the plasma membrane (to the right) are encircled. Elongation: An elongating particle is marked by an arrow. Integration: The labeled particles (corresponding particles are color-coded in successive frames) move toward the nucleus, fuse with each other, and integrate into the peripheral keratin network. Bundling: Inward-moving filaments fuse laterally and bundle (one example is marked by an arrow). Disassembly: The demarcated filament bundle disappears gradually without filament fragmentation. Bars, 2 µm.

turnover (Wöll et al., 2007; Sivaramakrishnan et al., 2009). Furthermore, the nonfilamentous keratin pool is increased during mitosis and in different stress paradigms, i.e., in situations of elevated network remodeling that are coincident with increased keratin phosphorylation (Chou et al., 1993; Liao and Omary, 1996; Omary et al., 1998; Strnad et al., 2002; Ridge et al., 2005). Interestingly, sumoylation has recently been implicated in keratin network dynamics (Snider et al., 2011).

Properties of the soluble keratin fraction

Heterotypic, nonfilamentous keratins most likely correspond to the biochemically defined soluble pool consisting of tetramers and/or small oligomeric assemblies (Soellner et al., 1985; Chou et al., 1993; Bachant and Klymkowsky, 1996). To prevent immediate assembly after biosynthesis or after filament disassembly, the soluble, nonassembled state must be stabilized, e.g., by protein modification, by association with chaperones such as Hsp70 and Hsc70, by interaction with IF-associated proteins (IFAPs), or by binding to 14-3-3 proteins (Liao and Omary, 1996; Wiche, 1998; Planko et al., 2007; Mashukova et al., 2009).

14-3-3 proteins predominantly bind to phosphorylated client proteins and are able to change their conformation (Kjarland et al., 2006; Díaz-Moreno et al., 2009). Therefore, it is an attractive assumption that Ser phosphorylation of keratin subunits along the head domain occurs soon after biosynthesis or disassembly to prevent assembly at nonpermissive sites in the cytoplasm. Hyperphosphorylation by Cdk1, Plk1, Rho-kinase, and Aurora B is important for local breakdown of several IF classes during mitosis and is essential for the efficient segregation of IF networks into daughter cells (Izawa and Inagaki, 2006). Given the comparatively small size of the disassembled subunits and their solubility in the aqueous cytoplasm, they should rapidly distribute throughout the cytoplasmic space by diffusion. A rapidly diffusible pool was recently identified by fluorescence recovery after photobleaching (Kölsch et al., 2010). Nucleation continues in the presence of actin filament and microtubule disruptors, which supports the notion that an active transport mechanism is not needed for delivery of keratins to peripheral nucleation sites (Wöll et al., 2005; Kölsch et al., 2009).

Regulation of the keratin cycle in space and time

The continuous transport of filamentous keratins toward the nucleus is essential for keratin cycling. Keratin isotype, cell type–specific properties, and other factors such as filament-associated proteins appear to determine and modulate this process. Growing keratin particles move preferentially along actin stress fibers at ∼300 nm/min (Wöll et al., 2005; Kölsch et al., 2009). This movement may be directly coupled to lamellar actin treadmilling through plectin-mediated linkage (Litjens et al., 2003; Rezniczek et al., 2004). Moreover, keratin particles can also be transported along microtubules (Yoon et al., 2001; Liovic et al., 2003; Wöll et al., 2005; Windoffer et al., 2006).

The molecular mechanism of the subsequent inward-directed movement of the keratin network (Windoffer and Leube, 1999; Yoon et al., 2001; Kölsch et al., 2010) is currently not clear. One possibility is that the intrinsic elasticity of the filaments (Kreplak et al., 2008) in combination with their nuclear anchorage, through the interaction between plectin and the outer nuclear membrane protein nesprin-3 (Wilhelmsen et al., 2005), accounts for it. Alternatively, actin filaments and/or microtubules are also involved in this movement. It has been observed that energy depletion inhibits KF motility, which can be taken as an indication of an energy-requiring and motor protein–driven active process (Hollenbeck et al., 1989; Strnad et al., 2001; Yoon et al., 2001).

Focal adhesion–dependent nucleation is another major cycle determinant, at least in vitro. One factor that may be involved is the cytoskeletal cross-linker plectin, particularly isoform 1f that has been localized to focal adhesions and is capable of binding to keratins (Nikolic et al., 1996; Steinböck et al., 2000; Litjens et al., 2003; Rezniczek et al., 2003). Other candidate proteins that may modulate keratin dynamics are integrins, vinculin, metavinculin, talin, and zyxin–focal adhesion components that have been shown to bind to IFs (Kreis et al., 2005; Ivaska et al., 2007; Kostan et al., 2009; Sun et al., 2008a,b, 2010). Besides these structural components, focal adhesion–dependent signaling is likely involved in keratin nucleation. Among additional factors, PKCs and MAPK have been discussed (Omary et al., 1992; Ridge et al., 2005; Osmanagic-Myers et al., 2006; Akita et al., 2007; Wöll et al., 2007; Bordeleau et al., 2008, 2010; Sivaramakrishnan et al., 2009). Yet, their spatiotemporal interaction with keratin assembly at focal adhesions is still unknown.

Cell shape changes, which occur frequently in motile and dividing cells, are expected to increase cycling, whereas stably anchored cells in mature tissues need very little cycling. To account for the gradual transition between these extremes, regulators should exist for graded and locally restricted responses. In support, cycling of wild-type and mutant keratins are slowed down by p38 MAPK inhibitors, presumably by affecting keratin phosphorylation (Wöll et al., 2007). This provides a possible mechanism for the attenuation of keratin cycling through stress-induced signaling. Compartmentalization of kinases and phosphatases such as focal adhesion kinase, protein kinase C, and others may further support localized alteration of network configuration. Members of the plakin family (which link cytoskeletal networks) and of the plakophilin family that localize to adhesion sites are prime candidates to fulfill such organizational functions, as they contain keratin binding sites and affect kinase/phosphatase activity (Osmanagic-Myers et al., 2006; Bass-Zubek et al., 2009; Kostan et al., 2009; Bordeleau et al., 2010). It was also demonstrated that shear stress increased PKCζ-mediated phosphorylation of K18-S33, resulting in an elevated exchange rate of the KF network (Sivaramakrishnan et al., 2009). The relevance of signaling-dependent keratin phosphorylation for dynamic network organization is most evident in disease: autoantibodies from the skin blistering disease Pemphigus vulgaris have been shown to induce p38 MAPK-dependent keratin retraction (Berkowitz et al., 2005). Furthermore, toxic liver injury induced by the antifungal drug griseofulvin leads to increased keratin phosphorylation, elevated soluble keratins, and aggregate formation (Ku et al., 1996; Stumptner et al., 2001; Toivola et al., 2004; Fortier et al., 2010), which can be counteracted, at least in vitro, by p38 MAPK inhibitors (Nan et al., 2006).

Exit of filaments from the turnover cycle

Plasticity must be weighed against the stabilizing properties of the keratin network providing mechanical strength to resting cells. In cultured interphase cells, desmosome- and hemidesmosome-anchored filaments, as well as the perinuclear cage-like structures, are prominent examples in which stabilization prevails over dynamics (Fig. 3). Video 1 presents an example of the long-term stability of filaments surrounding the nucleus. Furthermore, desmosome-anchored filaments are more resilient than the rest of the KF network to disruption by the tyrosine phosphatase inhibitor vanadate (Strnad et al., 2002). In a stable tissue context, cycling may become less and less important as mechanical functions become more important.

An attractive idea is that anchorage-dependent mechano-sensing affects KF stability by altered interaction with proteins that modulate KF dynamics such as desmoplakin, BPAG1, plectin, periplakin, and epiplakin (Bornslaeger et al., 1996; Wan et al., 2004; Osmanagic-Myers et al., 2006; Boczonadi et al., 2007; Spazierer et al., 2008; Ishikawa et al., 2010). In the case of the perinuclear network, attachment to the nuclear envelope via the plectin–nesprin-3a connection (Wilhelmsen et al., 2005) may confer filament stabilization; in the case of junction-associated KFs, special keratins such as K80 (Langbein et al., 2010) and keratin-binding proteins such as desmoplakin, plakophilin, plectin, and BPAG1 may be involved in KF stabilization (Guo et al., 1995; Eger et al., 1997; Holthöfer et al., 2007; Kostan et al., 2009; Green et al., 2010). Although all these results clearly show that anchorage protects KFs against disassembly, differential filament turnover has not been examined in detail at the single bundle/filament level. Chemical and/or biophysical keratin modification may be the result of coupling KFs to the mechanotransducive systems and may lead to KF stabilization much like the phosphorylation of the tail domain of the IF glial fibrillary acidic protein (GFAP) that reduces turnover (Takemura et al., 2002). We suggest referring to the acquisition of this property as "maturation," which is also observed for the other cytoskeletal filaments (e.g., association with proteins, detyrosination
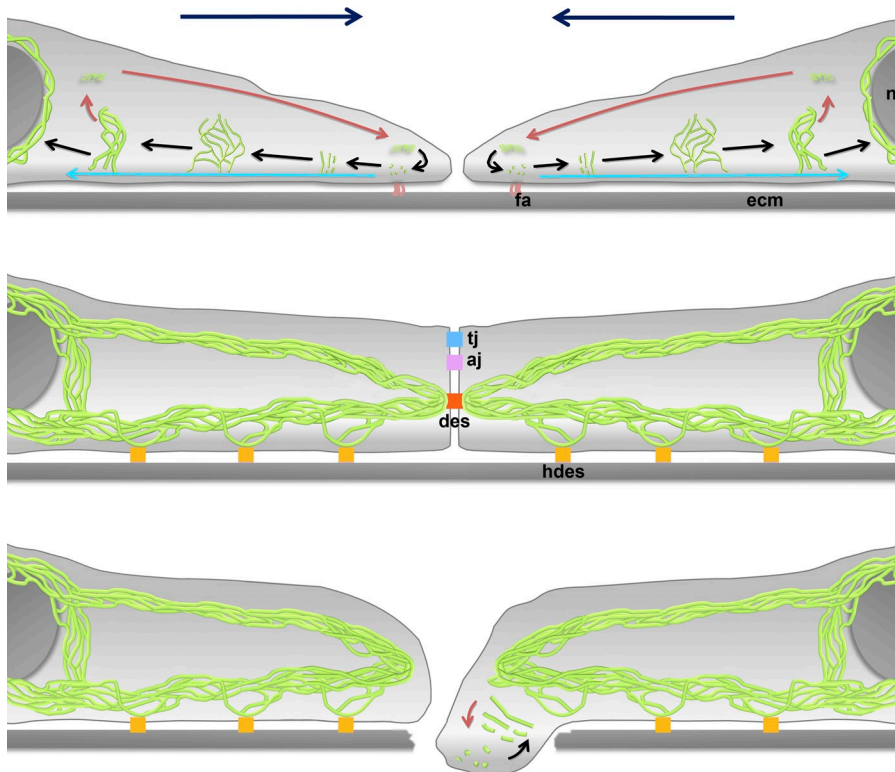
Figure 3. **Keratin network dynamics in three different situations.** (A) Approaching epithelial cells, e.g., upon wound closure: The motile cells require a dynamic cytoskeleton. This is supported by the keratin cycle, which allows rapid turnover and restructuring of the peripheral network using focal adhesion–dependent guidance cues, whereas the perinuclear network remains comparatively static. ecm, extracellular matrix; fa, focal adhesion; n, nucleus. (B) Differentiated static epithelium: Upon stable contact formation with neighboring epithelial cells through tight junctions (tj), adherens junctions (aj), and desmosomes (des), and with the extracellular matrix of the basement membrane through hemidesmosomes (hdes), the keratin network matures into topologically restricted thick bundles with very little keratin cycling. (C) Invasion of epithelial cell into connective tissue: Cells lose contact with each other and reduce stable hemidesmosomal adhesion coincident with up-regulation of keratin cycling in the leading edge for network remodeling.

of tubulin; Bulinski and Gundersen, 1991; Arce et al., 2008; Konishi and Setou, 2009; Ikegami and Setou, 2010).

Advantages of cytoskeletal cycling

Keratin recycling is certainly more efficient than degradation and de novo biosynthesis. Cycling has also been described for other cytoskeletal components, notably for the actin system characterized by filament treadmilling and retrograde flow in motile cells (Table I; Small and Resch, 2005; Schaus et al., 2007; Michalski and Carlsson, 2010). The dynamic instability of microtubules, i.e., the switching between growing and shrinking (Mitchison and Kirschner, 1984; Gardner et al., 2008), is another example for this strategy. Time-dependent, cyclic alterations of cellular components lead to diversification of functional states and, in turn, increase the probability for the cell to meet environmental demands by having the "right" response ready in time to ensure survival and functioning (Wolf et al., 2005; Vogel and Sheetz, 2009).

We suggest that cycling of keratins is a mechanism for checking the cell periphery for the occurrence of new cell contacts. Of note, the presence/absence of keratins affects the stability of desmosomes and hemidesmosomes (Long et al., 2006; unpublished data). In the case of focal adhesions, the cycle is accelerated by increased nucleation and thereby facilitates network growth toward the leading edge. In motile cells, the cycle may therefore be part of the complex assembly and disassembly mechanisms involved in moving the cell body (Proux-Gillardeaux et al., 2005). Upon filament attachment to hemidesmosomes and desmosomes, the cycle is slowed down and therefore supports continued mechanical stability. This mechanism may contribute to the peculiar in vivo distribution

patterns such as the localization in the terminal web, a dense filamentous network below the apical surface of the polarized epithelial cells of the gut (compare Oriolo et al., 2007). Collectively, keratin cycling may be interpreted as a continuous probing of the immediate extracellular surroundings until new physical contacts with other cells and/or the extracellular matrix can be established and "hard-wired" through desmosomes and hemidesmosomes. Because it does not require protein biosynthesis, it provides the cell with a variety of options to respond to environmental challenges within a small time frame. Thus, it is the dynamics of KF networks that enhances the adaptability and, hence, the function of moving cells.

Another important function of keratin cycling is to guarantee continued maintenance of an intact network during epithelial differentiation, which supports gradual polypeptide exchange without filament disruption. Thus, different admixtures of keratins are observed in basal versus suprabasal epidermal keratinocytes, and, consequently, basal type keratins are still detectable in cells that lack the corresponding mRNAs (Lersch and Fuchs, 1988; Reichelt et al., 2001).

Modulation of epithelial functions by keratin cycling

KFs have been regarded as a rather static component of the cytoskeleton conferring mechanical strength onto epithelia. This property is essential for resting cells, which provide the epithelium with mechanical strength. Loss of KF network integrity and, consequently, of epithelial rigidity leads to skin blistering disease (Coulombe et al., 2009). Yet, KFs also play a major role in dynamic processes, especially in wound healing and cancer metastasis (Paladini et al., 1996; Mazzalupo et al.,

Table I. **Basic assembly features of cytoskeletal filaments**

Properties	IFs	Microtubules	Actin filaments
Assembly units	Apolar fibrous tetramers	Globular heterodimers	Globular monomers
Nucleotide requirement	None	GTP	ATP
Nucleation sites/nucleating factors	Cell periphery/unknown	Microtubule organizing center (MTOC)/γ-tubulin ring complex	Juxtamembranous sites/ARP2/3 complex, formins
Elongation	Both ends	Plus end	"Barbed" end
Protofilaments	8	13-15	Double helix
Filament diameter	10 nm	25 nm	6 nm
Preferred disassembly site	Unknown	Minus end	"Pointed" end
Network remodeling	Lateral subunit exchange/ network cycling	Dynamic instability/treadmilling	Retrograde flow/treadmilling

The table summarizes textbook knowledge integrating also recent results on keratins. For further reading see, e.g., Chhabra and Higgs (2007), Herrmann et al. (2007), Akhmanova and Steinmetz (2008), Wade (2009); Campellone and Welch (2010).

2003; Knösel et al., 2006; Ptitsyn et al., 2008; Karantza, 2011). The keratin cycle, which proceeds at various scales ranging from diffusible filament precursors to macromolecular network components and spans the entire cytoplasmic space, may be a major contributing factor (Fig. 3).

An important prediction is that keratin cycling determines epithelial motility, migration, and vesicle trafficking, and that it is an important basis of the epithelial stress response. Regulation of cycling is linked to keratin modification, notably phosphorylation, and is therefore targeted by signaling pathways. A provocative but testable idea is that the predisposition of transgenic mice with phosphorylation-deficient K8 and K18 mutants to liver disease (Ku and Omary, 2006) and, conversely, the recently described cytoprotective effects of glycosylated keratins (Ku et al., 2010), are coupled to differences in keratin dynamics. The extent to which keratins regulate protein biosynthesis via 14-3-3 proteins or glucose transporters is likely to be affected by keratin dynamics (Kim et al., 2006; Vijayaraj et al., 2009). The activity of 14-3-3 proteins depends on target protein phosphorylation and nucleo-cytoplasmic distribution (Mackintosh, 2004). Expression of K17 (and possibly additional type I keratins) recruits 14-3-3 to the cytoplasm, where it stimulates the mammalian target of rapamycin (mTOR) pathway (Kim et al., 2006). The presence of keratins ensures the correct localization and function of the glucose transporter GLUT, with the absence of keratins leading to impaired glucose uptake and an AMPK-mediated down-regulation of mTORC1 (Vijayaraj et al., 2009). Finally, the degree of translational stimulation through elongation factor 4A (eIF4A)–plakophilin-1 may be regulated through the interaction of keratins with plakophilin-1 (Wolf et al., 2010). An important question is whether the distinct keratin assembly forms (e.g., filamentous vs. nonfilamentous) fulfill specific regulatory functions. Even more, one can predict that not only filamentous subdomains fulfill specific roles but that also nonfilamentous keratins act in a context-specific manner.

A further prediction is that accumulation of distinct cycle intermediates results in functional consequences on epithelial cell behavior. In support, cells lacking keratins or producing mutant keratins, which are characterized by reduced KFs and increased soluble keratins, migrate faster in scratch assays (Morley et al., 2003; Long et al., 2006).

Cycling in other IF systems

Other IF networks also appear to exchange subunits with mechanisms similar to those observed for keratins (Tsuruta and Jones, 2003; Mignot et al., 2007; Burgstaller et al., 2010). Extensive evidence has been provided for intense cross-talk between vimentin IFs and focal adhesions (Seifert et al., 1992; Gonzalez et al., 2001; Tsuruta and Jones, 2003; Spurny et al., 2008; Bhattacharya et al., 2009; Burgstaller et al., 2010), and plectin 1f has been shown to be instrumental in the recruitment of growing vimentin particles to focal adhesions (Burgstaller et al., 2010). Particle elongation has been examined for vimentin and neurofilament IF proteins in an elegant study demonstrating that it occurs by end-to-end annealing (Colakoğlu and Brown, 2009). Furthermore, cytoplasmic transport of filament precursor particles has been observed for various IF types (compare with Brown, 2003; Helfand et al., 2004; Wöll et al., 2005; Barry et al., 2007; Kölsch et al., 2009). But differences exist: although actin filaments appear to have a major role for keratin particles, microtubules seem to be more relevant for other IF types, such as vimentin, peripherin, and neurofilaments (e.g., Roy et al., 2000; Wang et al., 2000; Yoon et al., 2001; Helfand et al., 2003). Bundling has been explored for neuronal type IV IFs. In this instance, highly phosphorylated IFs are tightly packed in the center of the axoplasm, whereas less phosphorylated and more dynamic IFs are localized in the peripheral axoplasm (Sihag et al., 2007; Kushkuley et al., 2009).

Cycling of the IF cytoskeleton is likely important for many cell types, e.g., in neurons for growth cone probing (Chan et al., 2003), in mesenchymal cells and astrocytes for motility (Eckes et al., 1998; Lepekhin et al., 2001; Nieminen et al., 2006; Pan et al., 2008), or in endothelial cells for fluid shear stress adaptation (Helmke et al., 2001). Furthermore, IF cycling is probably needed for regeneration, e.g., upon neuronal axon injury, or in various stress situations, as is the case in reactive gliosis (Pekny and Lane, 2007).

Probably the strongest evidence for the importance of IF cycling stems from observations in *C. elegans*, where inhibition of sumoylation resulted in reduced IF network turnover coincident with increased IF bundling and aggregation (Kaminsky et al., 2009). It was further demonstrated that IF phosphorylation is linked to network organization and that both are coupled to hemidesmosome-mediated mechanotransduction (Zhang et al., 2011).

Conclusions and open questions

Recent observations support the view that plasticity of the epithelial KF network not only relies on changes in biosynthesis and degradation but is also accomplished by cycles of assembly and disassembly. Although this concept is supported by microscopic observations in cultured living cells, the underlying molecular mechanisms still need to be worked out in vivo and need to be correlated with observations on in vitro KF assembly (Herrmann et al., 2002) and alternative concepts of IF network dynamics (Ngai et al., 1990; Miller et al., 1991; Chang et al., 2006). Pertinent questions to be answered are: What powers the cycle? How do associated proteins such as plectin, 14-3-3, Akt-1, Hsp70, and others affect it? How do environmental factors such as mechanical force, cytokines, or microbes affect the keratin cycle, and what effects does the cycle have on the cell? How is KF cycling affected by keratin isotypes? What are the precise consequences of keratin modification? Which cellular processes are linked to keratin cycling and how is this accomplished? Is cycling needed for the stress protective function of keratins? And finally, how universal is the concept of cycling, and is it relevant to other IFs?

Online supplemental material

Video 1 is available at http://www.jcb.org/cgi/content/full/jcb .201008095/DC1.

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